ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LIX 27 Number 3, 2011

OCCURRENCE AND ANTIMICROBIAL SENSITIVITY IN STAPHYLOCOCCI ISOLATED FROM GOAT, SHEEP AND COW'S MILK

M. Vyletělová, O. Hanuš, R. Karpíšková, Z. Šťástková

Received: November 18, 2010

Abstract

VYLETĚLOVÁ, M., HANUŠ, O., KARPÍŠKOVÁ, R., ŠŤÁSTKOVÁ, Z.: *Occurrence and antimicrobial sensitivity in staphylococci isolated from goat, sheep and cow's milk*. Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 3, pp. 209–214

The aim of this study was to compare the sensitivity to selected antibiotics in staphylococci isolated from goat (n = 60), sheep (n = 60) and cow's milk (n = 120). The individual milk samples were inoculated onto Blood agar cultivated at 36 °C/24 h.The isolated species of staphylococci were identified using biochemical tests, namely STAPHYtest and identification program TNW pro 6.5. We examined the sensitivity of strains to the spectrum of antibiotics, as follows: vancomycin (VA), amoxicillin/clavulanic acid (AMC), penicillin (P), rifampicin (RD), oxacillin (OX), tetracycline (TE), erythromycin (E), chloramphenicol (C), clindamycin (DA), gentamicin (CN), ciprofloxacin (CIP), teicoplanin (TEC), cefoxitin (FOX) and novobiocin (NOV). Altogether, 97 staphylococcal isolates were obtained; 70 from cow's milk, 11 from goat's milk and 16 from sheep' milk. *Staphylococcus aureus* was the most frequent species in milk of all animal origin tested, was detected in 54 (45%) cow's milk, 10 (17%) goat's and 15 (25%) sheep' milk samples. *S. lentus* was identified only in goat's and sheep' milk whereas in cow's milk there were representation of staphylococcal species as follows: *S. haemolyticus* (n = 7), *S. chromogenes* (n = 2), *S. warneri* (n = 2), *S. xylosus* (n = 2), *S. epidermidis* (n = 2) and unclassified staphylococci (n = 1). The results of *S. aureus* sensitivity are similar for all tested antibiotics and for all monitored milk: No resistance to vancomycin, rifampicin, chloramphenicol and teicoplanin was recorded in obtained *S. aureus* isolates whereas the resistance to ciprofloxacin was found out most often.

milk, *Staphylococcus* spp., antibiotics

Quality of raw milk is closely related to production disorders in animals such as mastitis. Milk obtained from the infected udder is significantly of lower quality than that of the uninfected cows. Mastitis causes the changes in somatic cell count and *N*-acetyl-ß-D-glucosaminidase activity which are significantly higher in the infected udder. Lactose concentration is lower, whey protein and albumin concentrations are significantly higher (Leitner *et al*., 2004).

Staphylococcus aureus forms the constituent part of microorganisms which participate in mastitis pathogenesis and plays the most important role (in animal and human medicine) among all the species of *Staphylococcus* genus. In study by Vyletělová *et al*. (2010), the occurrence of *S. aureus* was observed within 10 years and it was found out that *S. aureus* (together with *Streptococcus uberis)* persists as the main pathogen causing mastitis. Wilson and Richards (1980) indicated 77% of positive findings of *S. aureus* in subclinical mastitis in cows in Great Britain. The same results (77% positive findings of *S. aureus*) were reported in Neetherlands (Grootenhuis, 1976).

Roberson *et al*. (1994) performed the ecological study of *S. aureus* at a cow breeding farm. In this study *S. aureus* was occurred in the whole dairy chain within the farm (dairy cows, bedding, air, insect, staff, milking system etc.), the highest risk for transfer of this bacteria are posed by the mammary gland and the animal body skin. In addition to the

findings of *S. aureus* other isolates of coagulase-negative staphylococci (CNS) were recorded, especially *S. haemolyticus*. In study carried out by Pitkälä *et al*. (2004) 4 237 positive quarter samples (from total number 12 661 samples – 3 282 cows) were confirmed and CNS were identified in 2 103 samples (49.6%).

The aim of this study was to compare the occurrence of staphylococci in raw milk of caw, goat and sheep origin and its resistance to selected antimicrobial agents.

MATERIALS AND METHOD

Herd characterization and milk sample collection

Individual milk samples were collected from one goat herd (W; White short-haired breed; n = 60), one sheep herd (C; Tsigai breed; n = 60) and one farm keeping two herds of Holstein cows (n = 50 and 70) were used in this study. Goats and sheep were bred in free stables with separate milking parlour and were kept in one farm which was located in the less favorable area in the Czech Republic. The milk samples from these small ruminants were collected from all animals in lactation in herds. Holstein cows were bred also in the free stables with separate milking parlour and samples were collected from animals with clinical (n = 2) and mostly subclinical (n = 117) mastitis.

Staphylococci detection and identification

Milk samples (0.05 ml) were inoculated onto Blood agar (Oxoid, UK) and cultivated at 36 °C for 24 h. Suspect colonies were isolated again on the Blood agar, cultivated at 36 °C for 24 h and identified biochemically using the STAPHYtest and identification program TNW Pro 7.5 (Pliva-Lachema). In the case of doubtful results strains were sent to the Czech Strain Collection of Microorganisms in Brno for more accurate identification (using specific biochemical tests or molecular methods).

Antibiotic susceptibility of *S. aureus* isolates

For antimicrobial susceptibility disc diffusion method has been used with antibiotic discs (Oxoid) as follows: vancomycin, amoxicillin/clavulanic acid, penicillin, rifampicin, oxacillin, tetracycline, erythromycin, chloramphenicol, clindamycin, gentamicin, ciprofloxacin, teicoplanin, cefoxitin and novobiocin (Table I). Mueller Hinton Agar (HiMedia, India) was used for performing the assay. Restults were interpreted according to CLSI standard (Table I).

S. aureus isolates were confirmed by the multiplex PCR method for detection of the species specific fragment SA442 (Martineau *et al*., 1998) and *mecA* gene which encodes resistance to methicillin (Bosgelmez-Tinaz *et al*., 2006).

RESULTS AND DISCUSSION

List of *Staphylococcus* species detected in milk samples is shown in Table II. In total, 97 isolates of staphylococci were identified. *Staphylococcus aureus* was the most frequent species in milk of all origin. *S. aureus* was found in 45% of examined cow's milk samples. Goat's milk and sheep'milk samples were positive for *S. aureus* in 17% and 25% respectively.

S. aureus is the main pathogen causing mastitis in cows in the Czech Republic (Vyletělová *et al*., 2010). *Staphylococcus aureus* is also the most frequently isolated from the clinical cases of mastitis in small ruminants (Bergonier *et al*., 2003; Vyletělová, 2009). Da Silva *et al*. (2004) detected that *S. aureus* represented 37% of the isolates from subclinical mastitis in goats and coagulase-negative

I: *List of antimicrobial agents tested and interpretation according to CLSI standard*

Antimicrobial agents	Symbol	Volume	Resistant	Intermediate	Sensitive
		(μg)	R	I	S
Vancomycin	VA	30	–	–	≥ 15
Amoxicilin/clavulanic acid	AMC	20+10	≤ 19	–	≥ 20
Penicillin	P	10	≤ 28	–	≥ 29
Rifampicin	RD	5	≤ 16	17–19	≥ 20
Oxacillin	OX	1	≤ 10	11–12	≥ 13
Tetracycline	TE	30	≤ 14	15–18	≥ 19
Erythromycin	E	15	≤ 13	14–22	≥ 23
Chloramphenicol	C	30	≤ 12	13–17	≥ 18
Clindamycin	DA	2	≤ 14	15–20	≥ 21
Gentamicin	CN	10	≤ 12	13–14	≥ 15
Ciprofloxacin	CIP	5	≤ 15	16–20	≥ 21
Teicoplanin	TEC	30	≤ 10	11–13	≥ 14
Cefoxitin	FOX	30	≤ 14	15–17	≥ 18
Novobiocin	NV	30	≤ 17	18–21	≥ 22

II: *List and frequency of individual staphylococci species detected in milk samples of various origin*

Species	Cows (n = 120)	Goats (n = 60)	Sheep (n = 60)
Staphylococcus aureus	54 (45%)	10 (17%)	15 (25%)
Staphylococcus haemolyticus	7	0	0
Staphylococcus chromogenes	2	0	0
Staphylococcus warneri	2	0	0
Staphylococcus xylosus	2	0	0
Staphylococcus epidermidis	2	0	0
Staphylococcus lentus	0	1	1
Other staphylococci.	1	0	0
Total	70 (58%)	11 (18%)	16 (27%)

III: *Antibiotic susceptibility of staphylococci from goat milk (sensitivity in %, without variable results)*

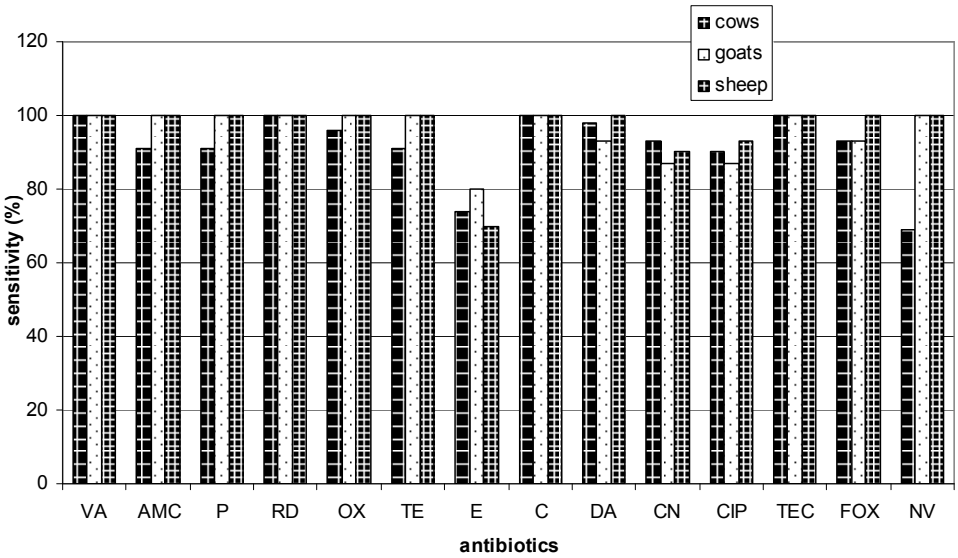
	n	VA	AMC	P	RD	OX	TE	E	C	DA	CN	CIP	TEC	FOX	NV
S. aureus	10	100	100	100	100	100	100	70	100	100	90	90	100	100	100
S. lentus	1	100	100	0	100	100	100	100	100	100	100	100	100	100	0

IV: *Antibiotic susceptibility of staphylococci from sheep milk (sensitivity in %, without variable results)*

	n	VA	AMC	P	RD	OX	TE	E	C	DA	CN	CIP	TEC	FOX	NV
S. aureus	15	100	100	100	100	100	100	80	100	93	87	87	100	93	100
S. lentus	1	100	100	0	100	100	100	100	100	100	100	100	100	100	0

V: *Antibiotic susceptibility of staphylococci from cow milk (sensitivity in %, without variable results)*

	n	VA	AMC	P	RD	OX	TE	E	C	DA	CN	CIP	TEC	FOX	NV
S. aureus	54	100	91	91	100	96	91	74	100	98	93	93	100	93	69
S. haemolyticus	7	100	71	71	100	71	86	71	100	86	71	86	86	71	71
S. chromogenes	2	100	100	100	100	100	50	100	100	100	100	100	100	100	100
S. warneri	2	100	100	50	100	100	100	100	100	100	100	100	100	100	100
S. xylosus	2	100	100	100	100	100	100	100	100	100	100	100	100	100	0
S. epidermidis	2	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Other staphylococci	1	100	100	100	100	100	100	100	100	100	100	100	100	100	100



1: *Comparison of sensitivity to antibiotics in Staphylococcus aureus from goat, sheep and cow milk*

staphylococci 60%. Contreras *et al*. (2007) identified also *Staphylococcus* spp. as the most prevalent pathogens in small ruminants and refer to necessity to eliminate this pathogen because of risk of milk contamination by thermostable toxins.

In study by Moroni *et al*. (2005) coagulase-negative staphylococci were reported to be the most common pathogens causing mastitis (accountable for 96% of infection). In our study other mastitis pathogens, e. g. *S. epidermidis* and *S. caprae* were not found out in milk samples, which is contrary to findings in studies by Contreras *et al*. (1995), Deinhofer and Pernthaner (1995), Moroni *et al*. (2005), in which *S. epidermidis* and *S. caprae* were te most often detected species in goats In our study next to *S. aureus* only *S. lentus* was found in goat's (n = 1) and sheep' milk (n = 1) samples whereas in cow's milk the distribution of staphylococcal species was as follows: *S. haemolyticus* (n = 7), *S. chromogenes* (n = 2), *S. warneri* (n = 2), *S. xylosus* (n = 2), *S. epidermidis* (n = 2) and other (unclassified) staphylococci (n = 1).

Results of antimicrobial susceptibility are shown in Figure 1. In total *S. aureus* originating from cow's milk were more resistant than those of goat and sheep origin. No resistance to vancomycin, rifampicin, chloramphenicol and teicoplanin was recorded in obtained *S. aureus* isolates. Two oxacillin resistant *Staphylococcus aureus* strains were detected originated from two dairy cows with subclinical mastitis, both were from one herd. Both were confirmed by PCR method as carriers of *mecA* gene.

CONCLUSION

The presence of resistant *Staphylococci* in basic food production poses a risk of spreading the pathogens to other animal species, humans involved in animal care and food processing, foodstuffs and consequently to the general population.

SUMMARY

This study was focused on comparison of the occurrence of staphylococci in raw milk of caw, goat and sheep origin and its resistance to selected antimicrobial agents. Individual milk samples were collected from one goat herd (W; White short-haired breed; n = 60), one sheep herd (C; Tsigai breed; n = 60) and from one farm keeping two herds of Holstein cows (n = 50 and 70). Milk samples were inoculated onto Blood agar and cultivated at 36 °C for 24 h. Suspect colonies were isolated again on the Blood agar, cultivated at 36 °C for 24 h and identified biochemically using the STAPHYtest and identification program TNW Pro 7.5. For monitoring of antimicrobial susceptibility disc diffusion method has been used, tested antibiotic discs were as follows: vancomycin, amoxicillin/clavulanic acid, penicillin, rifampicin, oxacillin, tetracycline, erythromycin, chloramphenicol, clindamycin, gentamicin, ciprofloxacin, teicoplanin, cefoxitin and novobiocin. *S. aureus* isolates were also confirmed by the multiplex PCR method for detection of the species specific fragment SA442 and *mecA* gene which encodes resistance to methicillin. Altogether, 97 staphylococcal isolates were obtained; 70 from cow's milk, 11 from goat's milk and 16 from sheep' milk. *Staphylococcus aureus* was the most frequent species in milk of all animal origin tested, was detected in 54 (45%) cow's milk, 10 (17%) goat's and 15 (25%) sheep' milk samples. Next to *S. aureus* only *S. lentus* was found in goat's (n = 1) and sheep' milk (n = 1) samples whereas in cow's milk the distribution of staphylococcal species was as follows: *S. haemolyticus* (n = 7), *S. chromogenes* (n = 2), *S. warneri* (n = 2), *S. xylosus* (n = 2), *S. epidermidis* (n = 2) and other (unclassified) staphylococci (n = 1). Results have been shown that *S. aureus* originating from cow's milk were more resistant than those of goat and sheep origin. No resistance to vancomycin, rifampicin, chloramphenicol and teicoplanin was recorded in obtained *S. aureus* isolates. Two oxacillin resistant *Staphylococcus aureus* strains (originated from two cows with subclinical mastitis) were detected and both were confirmed by PCR method as carriers of *mecA* gene. The presence of resistant *Staphylococci* in foods represents a risk for human and animal health and the risk of their cross-transfer.

Acknowledgements

This work was supported by Ministry of Agriculture project NAZV QH81111 and Ministry of Education projects MSM 2678846201, INGO LA 333, LA 10030 and LA 331.

REFERENCE

BERGONIER, D., DE CRÉMOUX, R., RUPP, R., GILLES, L. and BERTHELOT, X., 2003: Mastitis of dairy small ruminants. *Vet. Res*., 34: 689–716. ISSN 1993-5412.

BOSGELMEZ-TINAZ, G., ULUSOY, S., ARIDODAN, B. and COSKUN-ARI, F., 2006: Evaluation of different methods to detect oxacillin resistance in *Staphylococcus aureus* and their clinical laboratory utility. *Eur. J. Clin. Microbiol. & Infet. Dis*., 25, 410–412. ISSN 0934-9723.

CONTRERAS, A., CORRALES, J. C., SIERRA, D. and MARCO, J. C., 1995: Prevalence and etiology of nonclinical intramammary infection in Mariano-Granadina goats. *Small Rumin. Res*., (17): 71–78. ISSN 0921-4488.

CONTRERAS, A., SIERRA, D., SÁNCHEZ, A., CORRALES, J. C., MARCO, J. C., PAAPE, M. J. and GONZALO, C., 2007: Mastitis in small ruminants. *Small Rumin. Res*., (68): 145–153. ISSN 0921-4488.

DA SILVA, E., SIQUEIRA, A., MARTINS, J., FERREIRA, W. and DA SILVA, N., 2004: Identification and in vitro antimicrobial susceptibility of *Staphylococcus* species isolated from goat mastitis in the Northeast of Brazil. *Small Rumnin. Res*. 55, issues 1–13: 45–49. ISSN 0921-4488.

DEINHOFER, M. and PERNTHANER, A., 1995: *Staphylococcus* spp. as mastitis related pathogenes in goat milk. *Vet. Microbiol*. (43): 161–166. ISSN 0378-1135.

GROOTENHUIS, G., 1976: A randomized mastitis investigation in the Netherlands. *Report, Central Veterinary Institute*, Rotterdam.

LEITNER, G., CHAFFER, M., SHAMAY, A., SHAPIRO, F., MERIN, U., EZRA, E., SARAN, A. and SILANIKOVA, N., 2004: Changes in milk composition as affected by subclinical mastitis in sheep. *J. Dairy Sci*., 87: 46–52. ISSN 0022-0302.

MARTINEAU, F., PIKARD, F. J., ROY, P. H., OUELLETTE, M. and BERGERON, M. G., 1998: Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. *J. Clin. Microbiol*., 36**:** 618–623. ISSN 0095-1137.

MORONI, P., PISONI, G., ANTONINI, M., RUFFO, G., CARLI, S., VARISCO, G. and BOETTCHER, P., 2005: Subclinical mastitis and antimicrobial susceptibility of *Staphylococcus caprae* and *Staphylococcus epidermidis* isolated from two italian goat herds. *J. Dairy Sci*., 88: 1964–1704. ISSN 0022-0302.

PITKÄLÄ, A., HAVERI, M., PYÖRÄLÄ, S., MYLLYS, V. and HONKANEN-BUZALSKI, T., 2004: Bovine mastitis in Finland 2004 – prevalence, distribution of bacteria and antimicrobial resistance. *J. Dairy Sci*., 87: 2433–2441. ISSN 0022-0302.

ROBERSON, J. R., FOX, L. K., HANCOCK, D. D., GAY, J. M. and BESSER, T. E., 1994: Ecology of *Staphylococcus aureus* Isolated from Various Sites on Dairy Farms. *J. Dairy Sci*., 77 (11): 3354. ISSN 0022-0302.

VYLETĚLOVÁ, M., 2009: Identifikace mastitidních patogenů včetně MRSA v kozím a ovčím mléce a jejich citlivosti na antibiotika. *Výzkum v chovu skotu*, 4: 55–60. 0139-7265. ISSN 0139-7265.

VYLETĚLOVÁ, M., NEJESCHLEBOVÁ, L. and HANUŠ, O., 2010: Sledování hlavních mastitidních patogenů. *Náš chov*, 2: 68–71. ISSN 0027-8068.

WILSON, C. D. and RICHARDS, A., 1980: A survey of mastitis in the British dairy herds. *Vet. Rec*., 106: 431–435. ISSN 0042-4900.

Adresa

RNDr. MarcelaVyletělová, Ph.D., Výzkumný ústav pro chov skotu a Agrovýzkum Rapotín, s. r. o. , Výzkumníků 267, 788 13 Vikýřovice, Česká republika, doc. Ing. Oto Hanuš, Ph.D., Výzkumný ústav pro chov skotu, Výzkumníků 267, 788 13 Vikýřovice, Česká republika, doc. MVDr. Renata Karpíšková, Ph.D., MVDr. Zora Šťástková, Ph.D., Veterinární a farmaceutická univerzita Brno, Palackého 1–3, 612 42 Brno, Česká republika, e-mail: marcela.vyletelova@vuchs.cz